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Coagulase Negative Staphylococci Distribution in Dairy Herds with Automatic Milking System and their Crosstalk with *Staphylococcus Aureus* from IMI and Teat Apex



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BACKGROUND

- ❖ Longer milking duration and frequent exposure to teat disinfectants may affect the teat apex microbiota.
- ❖ Knowledge of epidemiological characteristics of CNS in automatic milking systems (AMS) herds is sparse
- ❖ The role of CNS on the risk to acquire of IMI with *S. aureus* is debated.

OBJECTIVES

- 1) To investigate the distribution of CNS species from aseptically collected quarter milk samples and teat skin in AMS herds,
- 2) To examine if the isolated CNS influence the expression of *S. aureus* virulence factors controlled by the *agr* quorum sensing system.

METHODOLOGY

- **Herd selection:** 8 herds: AMS with ≥ 3 milking robots and bulk tank PCR Ct-values ≤ 32 for *S. agalactiae*
- **Cows selection:** 30-40 cows/herd: SCC: $\geq 200,000$ cells/mL, and no antibiotic < 4 weeks prior to sampling
- **Quarter selection:** RH & LF quarters of cows with odd lab number (1,3,5..)
- Teat skin swabs (modified wet-dry method) and aseptic milk samples collected for bacterial culture using calf blood agar and SA SELECT™
- Suspicious CNS colonies were subjected to MALDI-TOF assay for species identification.
- Interaction between *S. aureus* and CNS investigated with Qualitative Beta-Galactosidase Reporter Plate Assay based on **3** reporter strains of *S. aureus*; **hla** (a-hemolysin), **RNAIII** (key effector molecule of *agr*) and **spa** (Protein A).

RESULTS

- ❖ 80% (228/284) quarters (142 cows) had ≥ 1 CNS species.
- ❖ 373 isolates, milk (n=105) and teat skin (n=268).
- ❖ 16 CNS species: teat skin (n=15), milk (n=10)
- ❖ Coinfections (mixed): 11 quarters from milk samples and 66 quarters from teat samples.
- ❖ Downregulation of *S. aureus* by some CNS may be explained by its ability to inhibit *S. aureus* *agr* system through production of auto-inducing peptide (AIP) molecules.
- ❖ Table 1 shows CNS species distribution and Figure 1 shows crosstalk between *S. aureus* and CNS

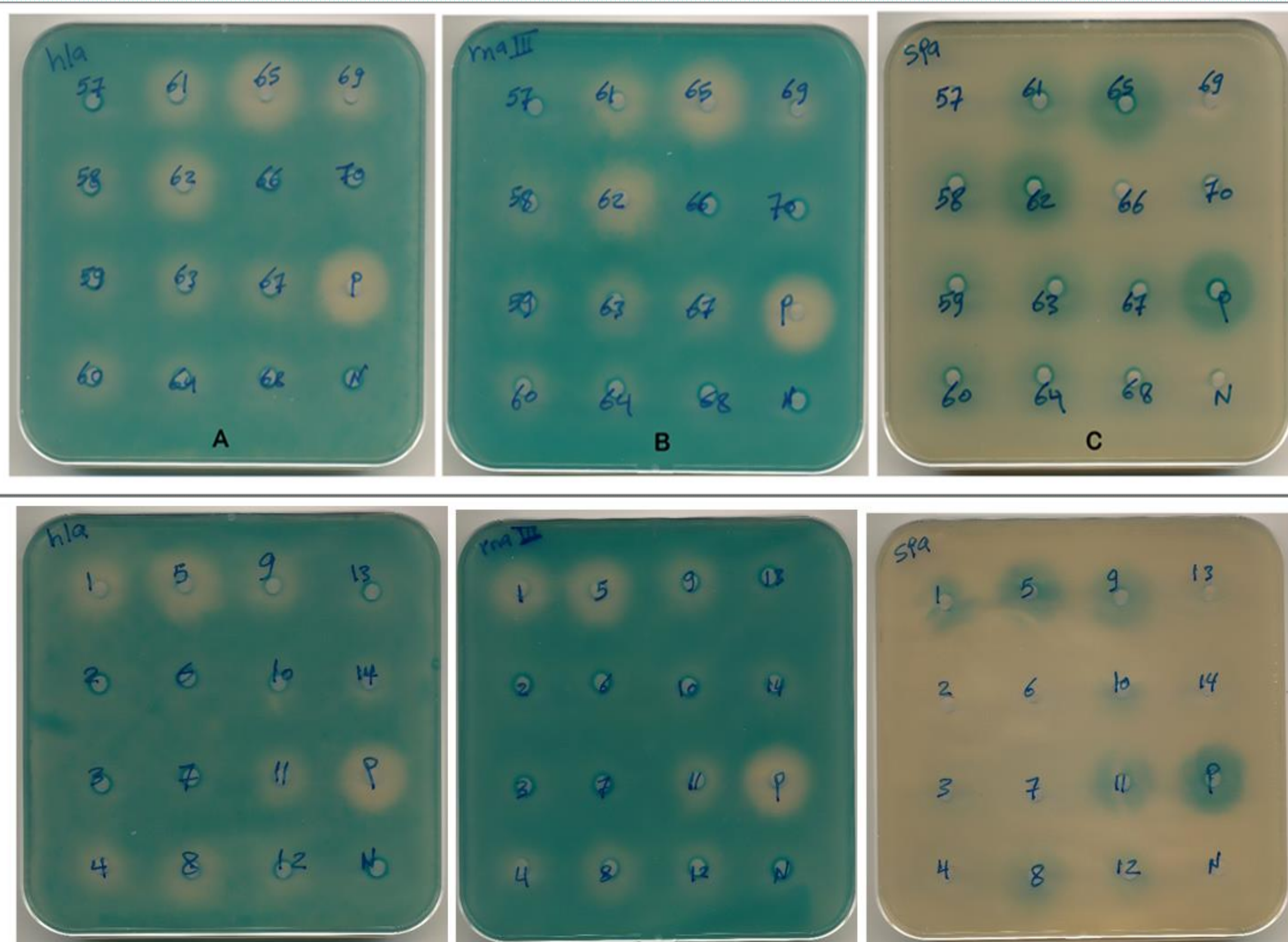
Table 1. Species distribution and association of CNS isolates from aseptic quarter milk and teat skin samples collected from 142 cows

CNS species	Sample type (%)		P-value
	Milk (n=105)	Teat (n=268)	
<i>S. arlettae</i> (12)	1 (0.9)	11 (4.1)	0.22 ^{NS}
<i>S. capitis</i> (3)	---	3 (1.1)	NA*
<i>S. chromogenes</i> (16)	11 (10.5)	5 (1.9)	0.002***
<i>S. cohnii</i> (43)	5 (4.8)	38 (14.2)	0.011**
<i>S. epidermidis</i> (60)	52 (49.5)	8 (3.0)	<0.0001****
<i>S. equorum</i> (122)	6 (5.7)	116 (43.3)	<0.0001****
<i>S. haemolyticus</i> (58)	16 (15.2)	42 (15.7)	0.90 ^{NS}
<i>S. hominis</i> (17)	3 (2.9)	14 (5.2)	0.48 ^{NS}
<i>S. piscifermentans</i> (2)	---	2 (0.8)	NA*
<i>S. saprophyticus</i> (5)	---	5 (1.9)	0.36 ^{NS}
<i>S. sciuri</i> (9)	---	9 (3.4)	0.13 ^{NS}
<i>S. simulans</i> (2)	2 (1.9)	---	NA*
<i>S. succinus</i> (2)	---	2 (0.8)	NA*
<i>S. vitulinus</i> (1)	---	1 (0.4)	NA*
<i>S. warneri</i> (2)	1 (0.9)	1 (0.4)	NA*
<i>S. xylosus</i> (19)	8 (7.6)	11 (4.1)	0.26 ^{NS}

CONCLUSIONS

- *S. epidermidis* and *S. chromogenes* are milk-associated, while *S. equorum* and *S. cohnii* are teat-associated.
- CNS species, habitat type, and herd factors affect CNS and *S. aureus* crosstalk patterns.
- Downregulation of *S. aureus* by some CNS species could explain possible protective effect.

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Downregulation effect

62, 65: *S. arlettae* (teat, H7)
61: *S. chromogenes* (teat, H7)
5: *S. chromogenes* (milk, H3)

Variant effect

1: *S. equorum* (milk, H1)
2: *S. equorum* (milk, H2)
6: *S. epidermidis* (milk, H3)
58: *S. epidermidis* (teat, H7)

No effect

66: *S. Sciuri* (teat, H7)
7: *S. Sciuri* (teat, H3)

Figure 1. Crosstalk patterns between *S. aureus* and CNS